Development of a Novel Prostaglandin EP_4 Agonist Which Stimulates Local Bone Formation *in vivo*

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INTRODUCTION

- Prostaglandin E_1 (PGE_1) and prostaglandin E_2 (PGE_2) stimulate bone formation both *in vitro* and *in vivo*
- Stimulation of the EP_2 and EP_4 receptor subtypes leads to bone formation
- Systemic EP_4 agonists decrease blood pressure – prevents use for general osteoporosis treatment but does not prevent their use as agents of local bone anabolism
- Current therapies for local bone formation – BMP-2 and BMP-7

Positives:
» Increased rate of bone formation
» Use in orthopedic and dental applications

Negatives:
» Biological molecules
» Expensive
» Storage conditions limit shelf life
» Required delivery system results in pulsatile systemic exposure
» Potential safety issues

Overall Aim:

Make a small molecule bone anabolic agent (EP_4 receptor agonist) with similar activities to the biological therapies currently available and and in doing so, address the negatives associated with their use

Project Goal:

- Produce a small molecule with:
  - » Reduced cost of goods
  - » Longer shelf life with ease of storage
  - » Single application in a slow release matrix
  - » Good local exposure but short half-life
    - Low systemic exposure

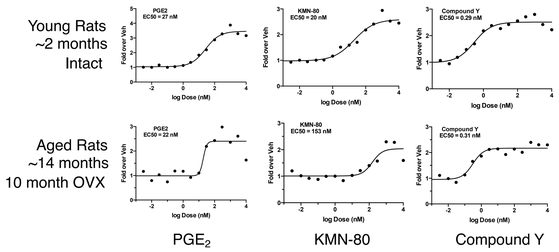
TABLE 1 EP_4 Binding and Functional (SEAP) Activity

	EP_1 Binding (nM)	EP_2 Binding (nM)	EP_3 Binding (nM)	EP_4 Binding (nM)
PGE_2	1.4	2.6	1.0	2.0
KMN-80	>10,000	>10,000	1,400	3.0
Compound X	>10,000	9,000	430	1.3
Compound Y	>10,000	120	400	0.74

	EP_2 Functional (nM)	EP_4 Functional (nM)
PGE_2	59	0.05
KMN-80	>1,000	0.19
Compound X	>1,000	0.04
Compound Y	394	0.01

Binding was determined by a [^{3}H]PGE_2 displacement/competition assay of human prostanoid $EP_{1\text{-}4}$ receptors in transfected HEK-293 cells (Cerep). Function activation was determined in HEK293 cells transfected with either rat EP_2 or EP_4 receptors together with secreted alkaline phosphatase (SEAP)-cyclic AMP response element (CRE) reporter constructs. SEAP activity was measured with a luminescence-based alkaline phosphatase substrate as a surrogate for cAMP formation.

FIGURE 1 EP_4 agonists convert bone marrow stem cells to osteoblasts in both young and old rats



Rat bone marrow was extruded from both tibia and femurs of either young or aged female rats. Cells were plated into 24-well dishes in MEMα supplemented with 15% FCS and cultured for seven days. One media change was done on day four (50% media withdrawn and replaced with an equal volume of fresh media containing $2x10^{-8}$ M dexamethasone ($1x10^{-8}$ M final). Alkaline phosphatase activity was quantitated by incubating 100 µl cell lysate with 50 µl pNPP substrate and reading product formation at 405 nm. EC_{50} values were determined from the data using GraphPad Prism. For the young animals, the experiments were repeated on at least 5 sets of rat, whereas a single group of aged rats has been used to this point.

FIGURE 2 Delivery modalities for different clinical environments

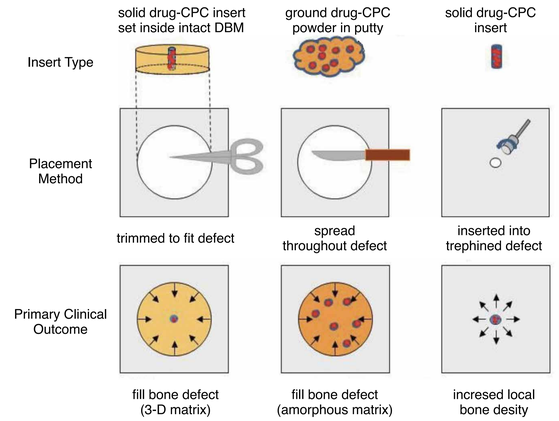
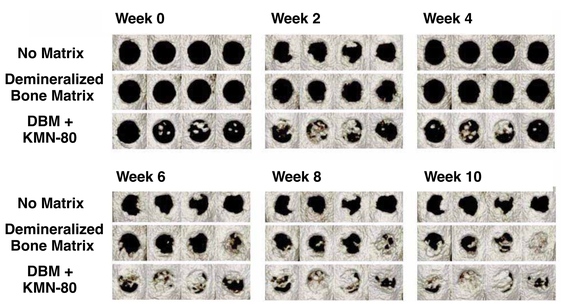
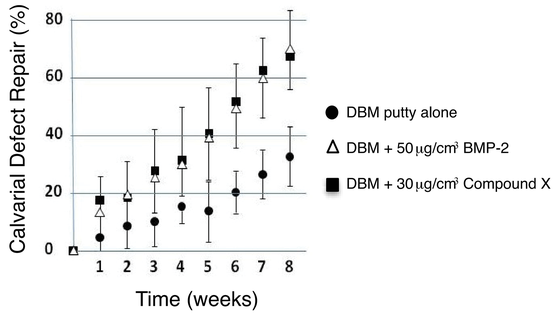


FIGURE 3 Calvarial Defect Repair - applications in orthopedics and reconstruction



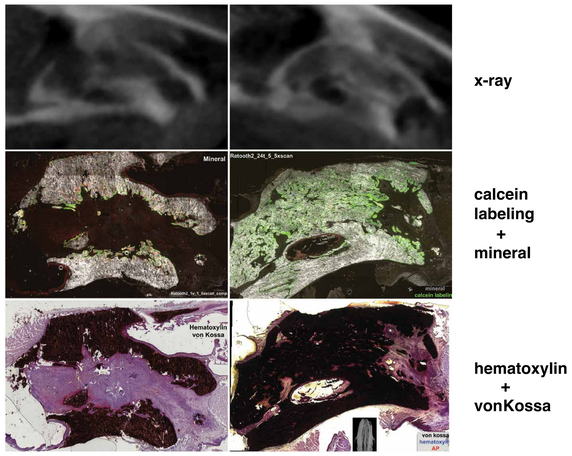
A 5 mm critical calvarial defect was treated with CPC inserts containing KMN-80 in solid demineralized bone matrix. Rats were imaged at weekly intervals and the size of the remaining defect measured from the cone-beam dental CT images (Vatech Pax Duo 3D).

FIGURE 4 Closure of a non-critical calvarial defect by EP_4 agonist or BMP-2



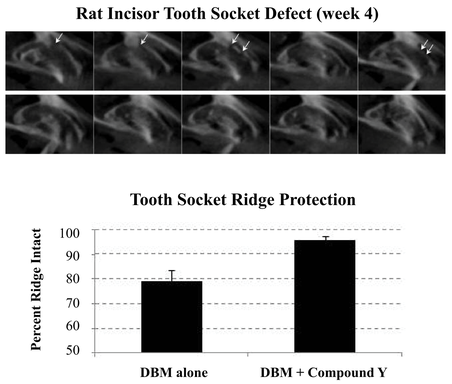
A 2.5 mm non-critical calvarial defect was treated with Compound X or BMP-2 suspended in demineralized bone putty matrix. Rats were imaged at weekly intervals and the size of the remaining defect measured from the cone-beam dental CT images (Vatech Pax Duo 3D). Defect size was calculated as percent repair compared to week 0.

FIGURE 5 Increased bone formation in a rat incisor tooth socket model - applications in dentistry



The right upper incisor was removed from 3 month old female SD rats. Following removal of the tooth, the socket was packed with demineralized bone matrix (DBM) putty alone (left panels) or DBM putty containing the novel EP_4 agonist KMN-80 (right panels) and bone formation was monitored over 10 weeks by cone-beam CT scanning. Calcein was injected two days prior to the end of the study to demonstrate areas of mineralization. After the final x-ray image 10 weeks post-treatment (top panels), the rats were euthanized and processed for histology by the lab of Dr. David Rowe (Univ. of Conn. Health Center). The middle panels demonstrate increased bone formation (calcein labeling) in the tooth socket when KMN-80 was included in the DBM putty and the bottom panels show the extensive formation of mineralized bone in the tooth socket of the same rat (von Kossa).

FIGURE 6 Buccal plate protection - applications in dentistry



The right upper incisor was removed from 3 month old female SD rats as in Figure 5. Following removal of the tooth, the socket was packed with demineralized bone matrix (DBM) putty alone (upper panels) or DBM putty containing compound Y (lower panels). Animals were imaged weekly to assess the extent of ridge remodeling; an event which occurs early following tooth removal and is often characterized by loss of bone from the ridge (white arrows). Shown here are the data at 4 weeks. Rats treated with standard therapy had between 71% and 93% of the ridge intact (mean = 79%) whereas rats treated with Compound Y had between 88% and 100% of the ridge intact (mean = 96%).

POTENTIAL THERAPEUTIC APPLICATIONS

Dental

- Alveolar ridge augmentation
- Periodontal disease-related bone loss
- Sinus lifts
- Dental implants

Reconstructive

- Distraction osteogenesis
- Craniofacial repair

Orthopedics

- Coating implants
- Augmentation of bone mass prior to implant
- Spinal fusion
- Repair of non-union fractures
- Bridging long bone critical defects (>3 cm)

CONCLUSIONS

- We have made a novel therapeutic agent for bone repair
- No observed toxicity
- Significantly lower cost of goods bringing with it a reduction in a major barrier to treatment
- Exhibits long term shelf-stability
- Slow release matrix allows single application